CHAPTER 3
MATERIALS AND METHODS

3.1 Study sites and historical backgrounds

Ethnobotanical fieldwork was conducted in 12 villages inhabited by four ethnic groups in the Nan province in northern Thailand. The precise location and basic information relating to each village are showed in Figure 2 and Table1.

Figure 2 Twelve villages in Nan province in northern Thailand where ethnobotanical fieldwork was conducted among four different ethnic groups

Table 1. Basic information for twelve villages in Nan province where ethnobotanical fieldwork was conducted among four ethnic groups

Village	Khang Ho	Manee Pruek	Song Khwae	Huai Labaoya	Huai Sanao	Santiphap
Ethnicity	Hmong	Hmong	Hmong	Mien	Mien	Mien
Geographic coordinates	19˚07’59.44”N 100˚56’00.38”E	19˚26’22.84”N 101˚04’11.35”E	18˚51’49.22”N 100˚38’08.69”E	18˚54’31.56”N 100˚40’05.30”E	19˚08’07.19”N 100˚56’02.79”E	18˚59’33.71”N 100˚39’16.50”E
Altitude (meters above sea level)	220	1,252	200	200	220	350
Number of households	333	236	373	142	188	61
Number of populations	1,968	1,299	1,818	615	934	425
Villager’s occupation	Farmer	Farmer	Farmer	Farmer	Farmer	Farmer
Presence of public health center	Yes	Yes	Yes	Yes	Yes	Yes
Presence of school	Yes	Yes	Yes	Yes	Yes	Yes
Nearby village(s)	Huai Sanao, Joon	Manee Pruek 2	-	Huai Pook	Khang Ho, Joon	-

Table 1. (continued)

Village	Huai Pook	Huai Satang	Nam Pan	Joon	Manee Pruek2	Toei Klang
Ethnicity	Khamu	Khamu	Khamu	Lua	Lua	Lua
Geographic coordinates	18˚53’52.81”N 100˚40’53.28”E	19˚32’03.64”N 100˚52’13.92”E	19˚26’14.49”N 100˚40’39.96”E	19˚08’40.35”N 100˚55’35.74”E	19˚24’53.21”N 101˚04’42.18”E	19˚13’38.03”N 101˚3’43.70”E
Altitude (meters above sea level)	220	300	350	220	1,280	850
Number of households	155	115	122	143	70	113
Number of populations	535	450	493	661	239	501
Villager’s occupation	Farmer	Farmer	Farmer	Farmer	Farmer	Farmer
Presence of public health center	Yes	Yes	Yes	Yes	Yes	Yes
Presence of school	Yes	Yes	Yes	Yes	Yes	Yes
Nearby village(s)	Huai Labaoya	-	-	Huai Sanao, Khang Ho	Manee Pruek	-

Originally, all the inhabitants of the villages settled in highland habitats. During the 1960–70s and until 1982, there was a conflict between the Thai government and the Communist Party of Thailand (CPT) which caused many families from the highlands to migrate (Jonsson, 2003; 2004). The CPT targeted remote provinces of strategic importance in the general area where our study sites are located. After CPT and their sympathizer occupied the mountains of northern Thailand, they influenced the ethnic minorities living there by offering services such as health care, education and other assistance to the poor (Rousset, 2009). At that time, there were widespread attacks by the Thai military on highland settlements inhabited by ethnic minorities, especially in Nan and Chiang Rai provinces where some regions were declared free-fire zones to be bombed and exposed to napalm (Jonsson, 2003). In order to isolate the highland population from the insurgents, a number of families from the highlands were abruptly herded to refugee camps and secure areas in the lowlands and their villages were abandoned or devastated.

Of the 12 villages investigated here, seven were affected by such conflicts and had to move to new settlements in lower lying areas. They include one Lua village (Joon) and one Khamu village (Huai Pook), two villages of the Hmong (Khang Ho and Song Khwae) and all three of Mien villages (Huai Labaoya, Huai Sanao and Santhiphap). The village of Joon, Huai Sanao, and Khang Ho are now located in the area called “Pla Klang” where there previously were refugee camps for those highlander families who were abruptly herded from highland habitats and subsequently established as permanent settlement. As for Huai Labaoya, Huai Pook, Santiphap, and Song Khwae, their populations also had fled from the interference of communists and settled in new areas at the present locations of the villages.

For those highlanders who had affiliated themselves to subversive activities in collaboration with the CPT and who later gave themselves up to the Thai government, some settled in new areas whereas others have remained in their old highland habitats. Two villages in this study; Manee Pruek and Manee Pruek 2, had joined the CPT. Nowadays, the village of Manee Pruek is still located in its original location whereas the village of Manee Pruek 2 has been moved from its original location near the Lua village, Toei Klang, to new area near Manee Pruek village of the Hmong.

Three villages, Nam Pan and Huai Satang of the Khamu and Toei Klang of the Lua, were not affected by the communist interference. These villages therefore remain in their original locations.

Nowadays, as a consequence of the abrupt move in the past, some villages of different ethnic groups are located next to each other. This is to say some villages of different ethnic groups occupying the same ecological environment. It is therefore taken as advantage for comparative purpose. Nearby village(s) for each village are also indicated in Table 1.

3.2 Qualitatively ethnobotanical fieldwork

3.2.1 Materials

3.2.1.1 Materials used for recording geographical information, taking photographs, and interview:

1) Altimeter

2) Camera, "Nikon Cool pix S7c"

3) Questionnaires

3.2.1.2 Materials used for specimen collecting:

1) Plastic bags and cutting tools

2) Tags or paper labels with string

3) Note books, pencils, rulers and permanent markers

3.2.1.3 Materials for plant pressing and chemicals for spirit collection:

1) Plant press 30x45 cm^2 with straps

2) Newspaper

3) Corrugated cardboards

4) Adsorbent papers

5) Hot Air Oven

6) 70% Ethyl alcohol and bottles for spirit collection

3.2.1.4 Materials for mounting herbarium specimens:

1) Mounting papers 30x 42 cm^2 in size

2) Paper labels 10x 13 cm^2 in size

3) Glue, needles and thread

4) White paper covers 60x 42 cm^2 in size

3.2.1.5 Materials used for plant identification:

1) Stereo microscope

2) Petri dish, needle and razor

3.2.2 Ethnobotanical field survey

Plant use data were collected between 2007 and 2011 in 12 villages, inhabited by ethnic groups. The duration of collecting qualitative ethnobotanical data in each village is at least one year round. Field surveys were conducted at least once a month or every other month in each season, during which ethnobotanical data were collected through interviews and group discussion (group interview) with the key and non-specialist informants. The key informants were deliberately selected among the local participants at each site based on their reputation as specialists with botanical knowledge, *e.g.,* folk healers, witch doctors, *etc.* Non-specialist informants are other villagers who were pleased to participate in the group discussion. By the technique of ethnobotanical inventory or field interview (Martin, 1995), all areas where a variety of useful plants were expected to occur, such as homegardens, cultivated areas, and nearby forest, were surveyed with the presence of two or more key informants. During the survey, the key informants/local participants were asked to indicate the useful plants and provide vernacular names and information as follows:

1. Plant part used: used parts (*i.e.*, leaves, infructescence, inflorescence, stem, root, bark, exudates, *etc.*),
2. Mode of preparation (*i.e.*, decoction, hot infusion, pounded, *etc*.),
3. Routes of administration (*i.e.*, poultices, teas, bath, *etc*.)

Vernacular name of plants and the ways they are used were exhaustively documented. Each time a plant was mentioned as used it was considered a single "use report" (Treyvaud Amiguet *et al*., 2005). For example, if plant A was used to treat fever, a single use record would be noted, however, if plant A was used to treat both fever and diarrhea, two use reports would be noted, and so on. Simultaneously, photographs of used plant was taken and their representative samples were collected in order to prepare herbarium voucher specimens and for the purpose of plant identification in the laboratory. The survey were conducted several times and stopped

when no more useful plants appeared; in other words, when a number of useful plants were repeatedly found.

Occasionally, after finishing the field survey, plant interviews together with group interviews (Martin, 1995) were conducted in each village with 5–8 non-specialist informants to enhance the precision of the findings during the survey. However, it was only possible to conduct the group discussion when an assemblage of villagers was encountered, usually, after coming back from the cultivated fields, when they assemble and relax by talking to neighbors.

After all field surveys were complete, to reconfirm the collected data, checklist interviews were conducted with groups of non-specialist informants. Plant pictures were also shown to those informants to avoid errors that could be caused by the variation of plant names. In addition to plant uses reported in the field survey by key-informants, some non-specialist informants from group interviews also provided information about additional uses of some plants that were not mentioned by the key informants. Of that knowledge, some is shared whereas most is idiosyncratic.

All plant uses reported by all informants were then classified into use categories following Cook (1995):

1. Food
2. Food additive
3. Animal food
4. Bee plants
5. Invertebrate food
6. Materials
7. Fuels
8. Social uses
9. Vertebrate poisons
10. Non-vertebrate poisons
11. Medicines
    - 11.1 Unspecified medicinal disorders
    - 11.2 Abnormalities
    - 11.3 Blood system disorders
    - 11.4 Circulatory system disorders

11.5 Digestive system disorders

11.6 Endocrine system disorders

11.7 Genitourinary system disorders

11.8 Ill-defined symptoms

11.9 Immune system disorders

11.10 Infections/Infestations

11.11 Inflammation

11.12 Injuries

11.13 Mental disorders

11.14 Metabolic system disorders

11.15 Muscular-skeletal system disorders

11.16 Neoplasm

11.17 Nervous system disorders

11.18 Nutritional disorders

11.19 Pain

11.20 Poisonings

11.21 Pregnancy/birth/puerperium disorders

11.22 Respiratory system disorders

11.23 Skin/subcutaneous cellular tissue disorders

12. Environmental uses

13. Gene source

3.2.3 Plant identification

It is important to know which plant species used and which vernacular name corresponds to which scientific plant name. Plant samples collected in the field are identified in order to ascertain the scientific nomenclature. Plant identification was based largely on the taxonomic literature such as *Flora of Thailand*, *Flora of China* (online: http://hua.huh.harvard.edu/china/mss/alphabetical_families.htm), *Flora of Java*, *Flora of British India*, *Thai Forest Bulletin*, and relevant available taxonomic revisions. Some common species were identified on site, using local names and/or a checklist of Thai plant names (Smitinand, 2001). The accuracy of plant identification was confirmed by comparison with herbarium voucher specimens from reference

herbaria, *e.g.*, Queen Sirikit Botanic Garden Herbarium (QBG) and the International Plant Name Index (www.ipni.org).

3.2.4 Preparation of herbarium voucher specimens (Giovannini, 2011)

Herbarium voucher specimens are very important because they provide long-term scientific record of a used plant. To prepare herbarium voucher specimens, plants collected in the field were dried, mounted on paper sheets and deposited in a herbarium. To dry plant specimens, the plant material is laid inside a folded newspaper sheet. Adsorbent papers are placed on both sides of each folded newspaper, and on each side of the adsorbent papers are placed corrugated cardboard. The stack of plant specimens are then pressed by using a plant press made of wooden frame. The plant press, stuffed with a pile of plant specimens, are then left near the source of heat; *e.g.*, hot air oven. Whenever the hot air oven is not available, *e.g.*, in the field sites, the sun was used as source of heat. By doing this, the plant press should be checked regularly and newspaper sheets must be regularly replaced in order to remove moisture and to dry the plants quicker. After drying, the specimens are mounted on special paper with glue, by sewing them, or with special tape. Once the plant species are identified, the labels can be prepared and attached to the herbarium sheets. In this study, herbarium voucher specimens of useful plants were deposited at the herbaria of the Ethnobotanical Research Unit, Department of Biology, Faculty of Science, Chiang Mai University and Queen Sirikit Botanic Garden Herbarium (QBG), Chiang Mai, Thailand.

3.3 Quantitative ethnobotanical study

3.3.1 Data analysis

All analyses, their elements, and their application, are explained in the Literature Review in Chapter 2.

3.3.1.1 The degree of agreement among informants for each use-category was assessed by calculating the Informant Consensus Factor (ICF). For those plants used as herbal medicine, ICF values were calculated separately for all sub-categories of use.

3.3.1.2 The importance of plant species mentioned as medicinal was assessed by using the Cultural Importance Index (CI).

3.3.1.3 The preference to medicinal plants used for particular purposes (ailments/disorders) was captured by using the Fidelity Level (FL).

3.3.2 Culturally important plant species and shared culture of plant uses

Because knowledge is "agreement between participants" and culture is "knowledge shared by a group" (Reyes-García *et al.*, 2003; Vandebroek, 2010), the CI values of used plants were therefore taken into account to determine the culturally important plant species. According to Heinrich *et al.* (1998), culturally important species are those that are used by a large number of people for the same category of use. Plants used as food and for other purposes, such as animal food, materials, and vertebrate poisons, may likely be more adapted to gathering environments. In contrast, traditional healing practices of a disease are closely related to cultural viewpoint of such disease. Therefore, the use of medicinal plants is considered being more culturally bound. Here, focusing on the uses of medicinal plants, the culturally important plant species of a particular ethnic group must be those that have high CI value (mostly those raised as domains for questionnaire interview) and their use related to specific medicinal purpose must be unique and shared among all three villages of a particular ethnic group.

The shared culture of plant uses by the ethnic groups studied was perceived from those medicinal plants which have high CI value and were reported for similar purpose of use by informants from a village of at least two ethnic groups. In other word, the similar uses of plants with high CI values reported from more than one village of different groups were considered as shared culture of plant uses among them.

3.3.3 Questionnaire interview

To determine prevalence of knowledge and use and to investigate the knowledge erosion, questionnaire interviews were applied to collect data related to knowledge and actual use of plants.

As, nowadays, the central concern related to traditional medicinal knowledge is that this valuable intellectual knowledge is gradually disappearing, possibly aggravated by expansion of modern healthcare systems (Ragupathy *et al.*, 2008). For this reason, the study of knowledge erosion focuses on traditional knowledge and actual use of medicinal plants. For each village, 30 medicinal plant species with high value of CI index were selected and raised as domain of questionnaire interview.

In total, 725 informants were randomly selected from local inhabitants (60-61 informants per village) based on gender and range of ages. Fifteen years old was considered as the cut-off age of acquisition to plant use knowledge (Ohmagari and Berkes, 1997; Reyes-García *et al.*, 2005). Therefore, only people over 15 years of age were interviewed. All interviews were stratified to six age groups (Table 2).

Each informant was asked about her/his knowledge and actual use of plants; for examples, "Do you know this plant and what its name is?" "Have you ever known/used this plant for medicinal purposes?" "How can it be used?" "How often do you use them?" "When did you use the plant last time?" During the questionnaire interview, pictures of the medicinal plants were shown to the informants in order to avoid misunderstanding (Thomas *et al*., 2007). The form of questionnaire used is demonstrated in Appendix B.

3.3.4 Study of knowledge erosion

Individual information of plant use knowledge and actual use of plants acquired from the questionnaire interview were statistically compared in order to investigate whether traditional plant use knowledge in each study site is undergoing erosion, and, if so, what the trend of knowledge erosion is.

The assumption was made, that the gap between knowledge and actual uses of plants can be used to study erosion of ethnobotanical knowledge. In this case, the loss of actual uses of plants was used as a proxy variable representing the loss of traditional plant use knowledge (Reyes-García *et al.*, 2005). As such, the null and alternative hypotheses for studying knowledge erosion are;

H_0: number of plants which medicinal knowledge related to them known to a given informant= number of those which were, or have been, actually used by such informant.

H_1: number of plants which medicinal knowledge related to them known to a given informant> number of those which were, or have been, actually used by such informant.

The Kolmogorov-Smirnov test was used for testing normality of data. In this regard, variables to be statistically analyzed for each village, number of medicinal plant known and actually used by each informant, did not have the normal distribution (Kolmogorov-Smirnov test; $p<0.05$); non-parametric methods were therefore applied for the statistical analyze in this study. For the dependent variables like number of known versus number of actually used medicinal plants, the *Wilcoxon Signed Ranks test* was used to test the hypothesis, whether there were significant differences between the known medicinal plants and those actually used.

3.3.5 **Prevalence of knowledge**

To investigate whether the knowledge and use of medicinal plants are prevalent among groups of informants, the Spearman's rank correlation coefficient was employed to determine correlations between subject variables; such as informants' age or educational level (years of formal education) versus number of known and actually used plants. All of the analyses were done with the SPSS 16.0 software package for Windows.

3.4 Comparative Study

All plants used by the four ethnic groups were compared intra- and inter-culturally. The comparisons of plant uses were conducted in the context of species diversity and species composition. In addition, comparisons of patterns of plants uses were conducted to investigate whether the use of plants by each ethnic group is influenced by cultural coherence or ecological divergence.

Table 2. Number of informants for questionnaire interview in twelve villages where ethnobotanical study was conducted

Ethnicity	Village	Number of informants for the questionnaires for each age range (females/males)						Total number
		15–19	20-29	30-39	40-49	50-59	>60	
Hmong	Khang Ho	10 (6/4)	10 (5/5)	9 (4/5)	10 (5/5)	10 (5/5)	11 (6/5)	60
	ManeePruek	9 (4/5)	11 (6/5)	10 (5/5)	10 (6/4)	10(5/5)	10 (5/5)	60
	Song Khwae	11 (5/6)	9 (5/4)	10 (5/5)	11 (6/5)	10 (5/5)	10 (5/5)	61
Mien	Huai Labaoya	10 (5/5)	10 (5/5)	10 (5/5)	11 (6/5)	10 (5/5)	11 (6/5)	62
	Huai Sanao	12 (7/5)	10 (6/4)	9 (6/3)	10 (6/4)	10 (5/5)	10 (5/5)	61
	Santiphap	9 (4/5)	9 (5/4)	10 (5/5)	11 (5/6)	11 (6/5)	11 (5/6)	61
Khamu	HuaiPook	7 (4/3)	10 (7/3)	10 (5/5)	12 (7/5)	10 (5/5)	12 (6/5)	60
	Huai Satang	10 (5/5)	10 (5/5)	10 (5/5)	10 (5/5)	10 (5/5)	10 (6/4)	60
	Nam Pan	9 (4/5)	10 (5/5)	10 (5/5)	9 (5/4)	10 (5/5)	12 (6/6)	60
Lua	Joon	10 (5/5)	10 (5/5)	9 (5/4)	10 (5/5)	10 (5/5)	11 (7/4)	60
	ManeePruek 2	9 (5/4)	14 (5/9)	12 (7/5)	11 (5/6)	9 (5/4)	5 (4/1)	60
	Toei Klang	10 (5/5)	10 (5/5)	10 (5/5)	10 (5/5)	10 (6/4)	10 (5/5)	60
	Total	116	123	119	125	120	123	725

3.4.1 Comparison of uses diversity

The diversity was estimated in the context of use diversity using use-category based Shannon-Wiener diversity index. The formula for the index was described in Chapter 2 – Literature Review. According to Begossi (1996), "pi" is the proportion between the number of citations for each species and the total number of citations. However, to remove the bias concerning the unevenness of number of informants to whom each species were showed, "pi" was adjusted to be the proportion between number of use-category reported for each species and the total number (summation) of use-categories reported for all species.

3.4.2 Comparison of species composition

Plant use data of each community were transformed into the presence/absence and the abundance matrices where species abundances were taken as the number of informants who cited the use(s) of particular plant species in such community. To compare species composition of plant used between villages and investigate whether plants used by all ethnic groups studied is similar, clustering technique, UPGMA cluster analysis, were applied to the entire set of plant uses by all villages. The UPGMA cluster analysis were performed using PC-ORD 6.0 package for Windows (McCune and Grace, 2002), based on data matrices and Jaccard's index of similarity.

In addition to comparison of entire set of plant species used by the four ethnic groups, the use of domesticated plants grown in their homegardens and the use of wild plants in the nature surrounding them were compared separately to investigate whether the use of plants by each ethnic group is culturally bound or is also a reflection of the ecological conditions under which they live.

3.4.2.1 Comparison of use of wild plants

Those plants found in natural surroundings of the villages were designated as wild plants. Similarity of the wild plant species used by each village was compared using UPGMA Cluster Analysis based on presence/absence data matrices and Jaccard's similarity index.

3.4.2.2 Comparison of use of domesticated plants

Homegardens are important site of experimentation and species domestication (Gautam *et al.* 2009). In addition to the gathering of wild plants and the cultivation of crops in fields, the four ethnic groups in this study establish homegardens and backyard plantings within and adjacent to their villages. As in homegardens elsewhere, their homegardens provide many kinds of plant used for food, medicines, materials as well as ornamentation.

In each village, all useful plants found in the anthropological areas were registered as domesticated plants. Additional 30–31 homegardens in each village were surveyed and homegarden owners were interviewed regarding their use and source of plants grown in their homegardens. To compare the composition of domesticated species between village of different and identical ethnic groups, the UPGMA Cluster Analysis was employed based on the presence/absence matrix and Jaccard's similarity index.

3.4.2.3 Comparison of plant use patterns

To investigate patterns of agreement and disagreement concerning a domain of knowledge among different or identical ethnic, sets of plants used for a particular use-category (Cook, 1995) in each community; *i.e.*, presence/absence matrix, were compared using UPGMA Custer Analysis based on Jaccard's similarity index.